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DEVELOPMENT AND VALIDATION OF DIFFERENCE SPECTROPHOTOMETRIC METHOD FOR THE ESTIMATION OF TENOFOVIR DISOPROXIL FUMARATE IN ITS PURE AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

A simple, precise and accurate difference spectroscopic method has been developed for the estimation of tenofovir disoproxil fumarate in its pure and pharmaceutical dosage form. In two sets of 10 ml volumetric flask, aliquots of standard drug solution in distilled water were transferred and diluted the first set with 0.01M sodium hydroxide and other set with 0.01M hydrochloric acid to get a concentration of 10-50 µg/ml for both the sets. The difference in absorbance of tenofovir disoproxil fumarate at 239 nm (maxima) and 224 nm (minima) was calculated. Beer's law is obeyed in the concentration range of 10-50 µg/ml with a linear regression value 0.998. As per ICH guidelines the results of the analysis were validated statistically and were found to be satisfactory. Recoveries obtained do not differ significantly from 100% showed that there was no interference from the common excipients used in the tablet formulation indicating accuracy and reliability of the method. The limit of detection and limit of quantification were found to be respectively.

KEYWORDS: Tenofovir disoproxil fumarate, UV visible spectrophotometer, Difference spectrophotometry, Hydrochloric acid, Sodium Hydroxide.

INTRODUCTION[1-5]

Tenofovir disoproxil fumarate (Fig. 1.), [[(2R)-1-(6-aminopurin-9-yl) propan-2-yl] oxymethyl-(propan-2-yloxycarbonyloxymethoxy) phosphoryl] oxymethyl propan-2-yl carbonate; (E)-but-2-enedioic acid, is a prodrug of neucleoside reverse transcriptase inhibitors that inhibit HIV replication during HIV transcription. It is widely used in the treatment of HIV infection and chronic hepatitis B.

Literature survey revealed that several UV- Visible spectrophotometric and HPLC work has been reported for the determination of Tenofovir disoproxil fumarate in pharmaceutical preparations. But no method has been reported yet to estimate the amount of the drug in presence of interference.

The objective of the present investigations was to develop simple, accurate and economical difference spectrophotometric method for estimation of tenofovir disoproxil fumarate in pharmaceuticals dosage forms. The essential feature of a difference spectrophotometric assay is that the measured value is the difference absorbance between the analyte in different chemical forms in two equimolar solutions, which exhibit different spectral characteristics.

The simplest and most commonly employed technique for altering the spectral properties of the analyte is the adjustment of the pH by means of aqueous solutions of acid, alkali or buffers.

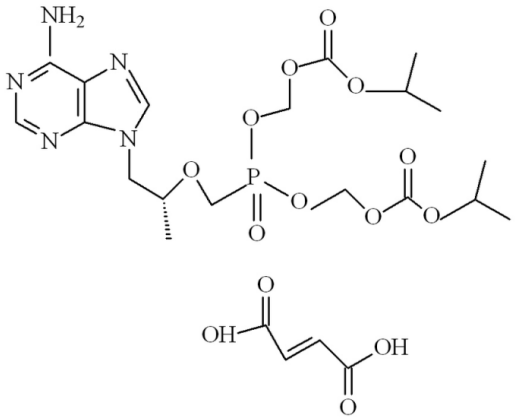

Fig. 1. Structure of tenofovir disoproxil fumarate.

MATERIALS AND METHODS

Materials

A SHIMADZU model PHARMASPEC-1800 UV Visible double beam spectrophotometer UV probe 2.24 with 1cm matched cuvette was used for the spectral measurements. Tenofovir disoproxil fumarate API was provided by Emcure pharmaceutical Ltd., (Hyderabad, India), as a gift sample. Sodium hydroxide and Hydrochloric acid were purchased from Spectrum Reagents and Chemicals Pvt. Ltd., Cochin. All the chemicals used were analytical reagent grade.

Preparation of standard stock solution

100 mg of tenofovir disoproxil fumarate API was weighed accurately and transferred to volumetric flask and made up to 100 ml (1000 µg/ml).

Preparation of working standard solution

10 ml of the stock solution (1000 µg/ml) was further diluted to 100 ml with distilled water to get a concentration of 100 µg/ml.

Preparation and analysis of tablet formulation

20 tablets were accurately weighed and triturate thoroughly to get fine powder. The powder equivalent to 100mg of tenofovir disoproxil fumarate was weighed and transferred in to 100ml volumetric flask. The content of the flask were dissolved in distilled water with the aid of ultra sonication for 5 minutes. The solution was filtered to through whatmann filter paper and the volume was made up to 100ml with distilled water. From the resultant solution, 10ml were pipetted and made up to 100ml with distilled water. 1ml of the solution were again pipetted into two 10 ml standard flask and make up to 10ml with 0.01M HCl and 0.01M NaOH separately to get final concentration of tenofovir disoproxil fumarate. The difference absorption spectrums are plotted with the drug in HCl solution in the sample cell relative to the drug in NaOH solution in the reference cell after the zero absorbance had been set with the 0.01M HCl in the sample cell and 0.01M NaOH solution in the reference cell. The difference in absorbance at 239 nm (maxima) and 224 nm (minima) was calculated.

Method Validation[6-8]

The method was validated according to ICH Guidelines. The validation parameter included are linearity, accuracy, precision, ruggedness, limit of detection and limit of quantification.

Linearity

Aliquot of working standard solution (1, 2, 3, 4 and 5ml) were pipetted into two sets of 10ml standard flasks to get 10–50 µg/ml concentrations. One set made up to 10ml with 0.01M HCl and other set with 0.01M NaOH. The difference absorption spectrums are plotted with the drug in HCl solution in the sample cell relative to the drug in NaOH solution in the reference cell after the zero absorbance had been set with the 0.01M HCl in the sample cell and 0.01M NaOH solution in the reference cell. The difference in absorbance at 239 nm (maxima) and 224 nm (minima) was calculated. Calibration curve was prepared by plotting concentration versus difference in absorbance.

Accuracy

The accuracy of the method was determined at the three percentage level 80%, 100%, 120%. The percentage recovery and relative standard deviation was found out.

Precision

To determine the precision of the proposed method, sample solution at a concentration within the working range were prepared and analysed in three replicates during the same day and on three consecutive days and results were found out.

Ruggedness

Ruggedness was determined by performing analysis of formulation following the recommended procedures by three different analysts.

Limit of detection (LOD) and Limit of quantification (LOQ)

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated. The quantitation limit of an individual analytical procedure is the lowest

amount of an analyte in a sample which can be quantitatively determined with suitable precision and accuracy.

LOD = 3.3 σ/S

LOQ = 10σ/S

Where σ is the standard deviation and S is the slope of the curve.

RESULT AND DISCUSSION

Analysis of marketed formulation

The assay was performed in triplicates and the values was shown in Fig.2 and Table 1

Table 1: Result for analysis of marketed formulation.

Marketed formulation	Drug	Label claim (mg)	Estimated amount (mg)	%purity	%RSD
TenoHep	Tenofovir disoproxil fumarate	300mg	294	98.03%	1.144
			300	100%	
			300	100%	

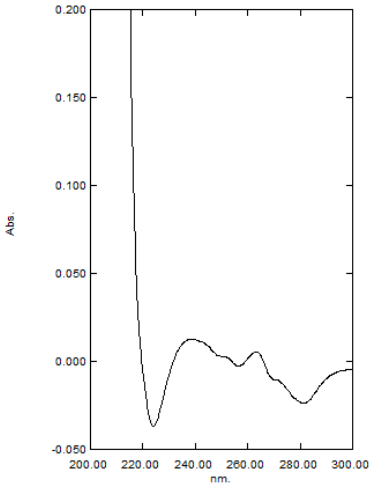

Fig. 2. UV Difference spectra of tablet formulation.

Method Validation

Linearity

The calibration curve of tenofovir disoproxil fumarate was linear in the concentration range of 10-50µg/ml. Overlay difference spectrum (Fig. 3) was taken. The calibration curve for tenofovir disoproxil fumarate was obtained by measuring the difference in absorbance at the λmax of 239nm and λmin of 224nm and values are shown in Table 2 and calibration curve were shown in Fig. 4. The correlation coefficient value obtained was 0.998.

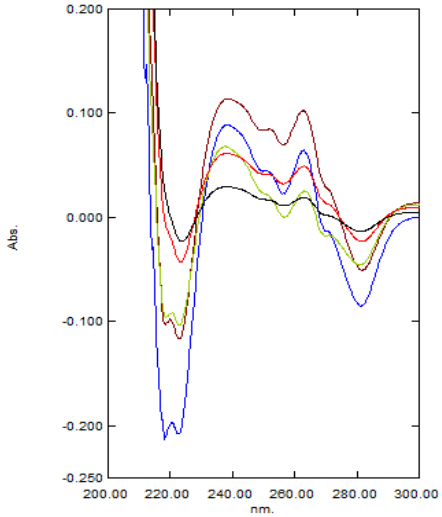

Fig. 3. Overlay difference spectra of Tenofovir disoproxil fumarate.

Table 2: Linearity of tenofovir disoproxil fumarate by difference spectroscopy.

Sr.NO.	Concentration (µg/ml)	Absorbance At 239nm	Absorbance at 223nm	Difference in absorbance
1	10	0.029	-0.022	0.046
2	20	0.061	-0.042	0.103
3	30	0.066	-0.098	0.164
4	40	0.114	-0.110	0.224
5	50	0.088	-0.194	0.282

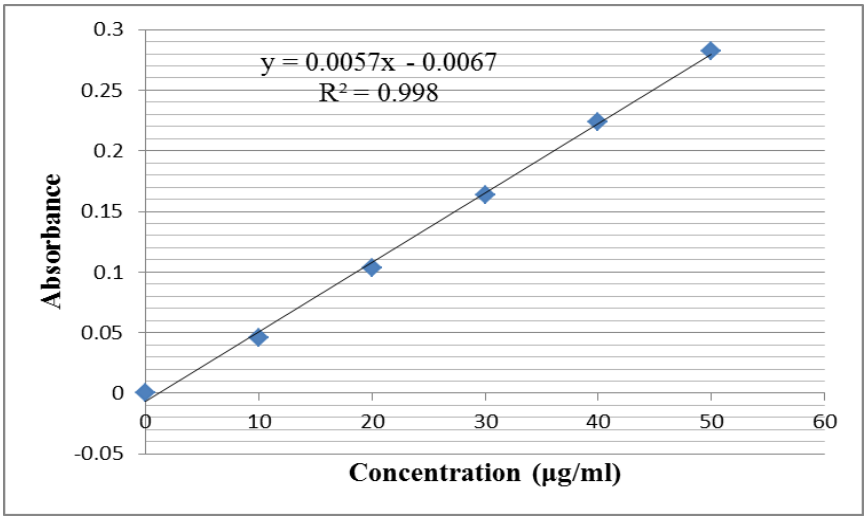

Fig. 4. Calibration curve of Tenofovir disoproxil fumarate.

Accuracy

To access the accuracy of the proposed method studies are carried out at three different target levels 80%, 100%, 120%. The recovery studies were carried out three times and the percentage recovery were within the limit and % RSD was found to be less than 2. The result are given in table 3.

Table 3: Result of accuracy study.

Drug	Theoretical % target level	Labelled claim	Amount of Drug Recovered (mg)	% purity	% RSD
Tenofovir disoproxil fumarate	80%	300mg	323	107.9%	1.05%
	100%		317	105.9%	
	120%		323	107.8%	

Precision

The precision of the method was determined by Intra-day and inter-day precision studies by taking 10µg/ml concentration of sample. Value of % RSD for intra-day found to be 1.14 and for inter-day were 1.102 respectively and the result are shown in Table 4.

Table 4: Result of precision study.

Drug	Amount (µ/ml)	Intraday Precision		Interday Precision	
		% Content	% RSD	% Content	% RSD
Tenofovir disoproxil fumarate	10	100	1.147	101.96	1.102
		98.03		103.92	
		98.03		101.96	

Ruggedness

Ruggedness was determined by performing the proposed method by three different analysts. The value of %RSD was found to be less than 2 and the results are shown in Table.5.

Table 5: Result of Ruggedness study.

Drug	Parameter Altered	Amount Taken (µg/ml)	Amount Recovered (g)	% Content	% RSD
Tenofovir disoproxil fumarate	Analyst 1	10	0.294	98.039	1.147
	Analyst 2		0.3	100	
	Analyst 3		0.294	98.039	

LOD and LOQ

The LOD and LOQ were calculated based on the standard deviation and slope of the curve and the result were shown in Table 6.

Table 6: Result of LOD and LOQ.

Drug	LOD(µg/ml)	LOQ(µg/ml)
Tenofovir Disoproxil Fumarate	3.87	9.84

Summary of Analytical data parameters are given in Table 7

Table 7: Analytical data parameters.

Sr. No	Parameters	Tenofovir disoproxil fumarate
1	λ max	239nm (λ max), 223nm (λmin)
2	Linearity Range	10-50 µg/ml
3	Regression equation	Y=0.0057x - 0.0067
4	Slope	0.0057
5	Correlation coefficient	0.998
6	LOD	3.87
7	LOQ	11.7

CONCLUSION

A simple, precise and accurate difference spectroscopic method has been developed for the estimation of tenofovir disoproxil fumarate in its pure and pharmaceutical dosage form. The developed method was found to be highly sensitive, specific and inexpensive at the same time. The method was validated as per the ICH guidelines. Hence the method stands validated and can be used for the routine quality control analysis of tenofovir disoproxil fumarate in tablet dosage form.

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